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CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS

EXPLORATIONS IN EXOBIOLOGY

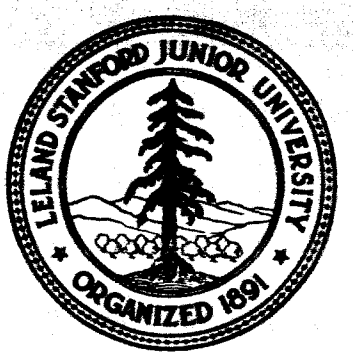
NASA CR 51096

Status Report Covering Period March 1, 1962 - April 1, 1963

For

National Aeronautics and Space Administration

Grant NsG 81-60



Instrumentation Research Laboratory, Department of Genetics

Stanford University School of Medicine

Palo Alto, California

Report* to the National Aeronautics and Space Administration

"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

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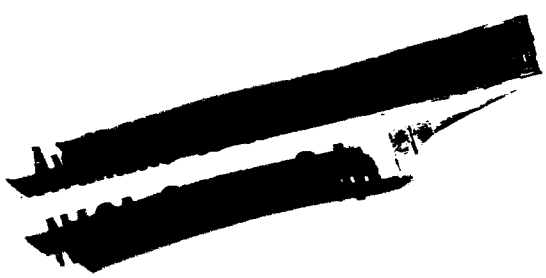

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* Parts of this report and the appendices are being prepared for publication, for which further editing is indicated. They are submitted in their present form, to make them available for use by NASA and cooperating laboratories at the earliest possible time.

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Removed for separate evaluation

1. Introduction

This report covers the period, March 1, 1962 to April 1, 1963, during which time the Instrumentation Research Laboratory was organized as part of the Department of Genetics at Stanford University. Its principal mission is the exobiology program. In addition we will seek to use the talents, specific skills and interests we have and will acquire in the future in connection with the exobiology activities to generate new programs in biomedical instrumentation in collaboration with various members of the university faculty. Efforts in this direction have already been initiated. Full implementation of this aspect of the laboratory activities will await completion of new facilities resulting from NASA grant NsG(F)-2.

The first part of this report considers the general problem of the quest for signs of life. The next outlines the basic concepts of the Multivator design philosophy and describes the present state of development. Also included, for the sake of completeness, is a brief review of the mechanical design being carried out as a separate program under the direction of Professor J. Arnold of the Mechanical Design Department.(1) The third part covers the work on particular assays being developed for inclusion in the Multivator. Following this we report on our efforts on scanning systems of several kinds. The last technical part of this review discusses our computer activities. The last section is on personnel and organization and includes a list of papers and seminars presented by members of the laboratory and a roster of the present staff. Appendices A through D provide a more detailed presentation of several aspects of our work.

2. Signs of life

The field of exobiology is growing both in the scope of its problem and in the number of scientists devoting attention to them. It therefore becomes a matter of importance to attempt to state what these concerns are in an organized way. This should serve to indicate where new experimental and theoretical effort is needed and to provide a framework for the evaluation of specific experimental possibilities as they arise.

There are an increasing number of reasons for accepting the possibility that life in the form of reasonably complex replicating molecules is prevalent throughout the universe. This same kind of reasoning leads one to further suppose that intelligent forms of life are perhaps an order of magnitude less prevalent. The quest for signs of life is the overriding immediate concern of exobiology and leads to different experimental criteria than the concern which follows; namely, understanding the nature of the life where signs have been discovered. Obviously the successes and even the failures of the search give some insights pertinent to the later analytical concern, but the instrumentation for search can be different than for analysis.

The difference between criteria for search and analysis is illustrated particularly clearly in connection with the question of intelligent life in other solar systems. At first one has to explore the complete domain of space and possible communication links, asking only for

evidence of non-random phenomena unexplainable by known physical laws. A positive response to such a query is not at all convincing as far as the question of the existence of intelligent life elsewhere and says nothing of its nature. It, however, rationalizes an enormous narrowing of the region of search and thereby allows a corresponding increase in the analytical possibilities.

Besides the distinction between search and analysis there is another meaningful classification. Efforts to find simple forms of life by direct contact will, for at least the next decade, be limited to our solar system, where there is no evidence for the existence of intelligent forms of life* on any planet other than the earth. Thus, for the present, the search outside the solar system is necessarily limited to intelligent forms of life capable of communication, and that within the solar system for simple forms.

The dichotomy between search and analysis enters into the search for signs of simple forms of life within our planetary system. This is sometimes

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* There may be other manifestations of well developed intelligence on earth other than human intelligence (i.e. Cetacae). If this is so, attempts to communicate with these species permit the development of analytical techniques independently of search techniques, and require no development of technology and manipulative skills on the part of the species with whom we are attempting to communicate.

obscured by the desire to avoid a two-step process and combine both functions in one experiment. This demands an experiment that is at the same time completely general and yet sufficiently rich in analytical details to give an unambiguously interpretable answer. This combined goal is sought not only because the prize of definitive proof of the existence of life elsewhere is so great, but also because of the problems associated with contamination. This latter concern puts a high premium on the first landed capsule giving an unequivocal answer. It also supports the conclusion that the first capsule should not be landed until fly-by search missions have served to narrow either or both the geometrical volume and the volume of a matrix of possible chemical and biological properties of living systems that must be analyzed.

It is such a matrix that needs to be established in order to have a basis for choosing experiments for either search or analytical missions.

A possible format for such a matrix is shown in fig. 1. The concept of this matrix will be clarified by the following comments.

(a) Here we list the elements common to terrestrial living systems.

(b) In this column the typical chemical structure and function remain the same, but there are substitutions within a class. For example, arsenic rather than phosphorus is used for formation of energy storage bonds; or selenium or sulphur is used to play the role of oxygen; alpha aminobutyric acid might be an amino acid.

(c) Under remote analogues we simply specify inorganic, organic, and macro-molecules that play functional roles similar to those found in the terrestrial system but do not ask that they have the same structure, for example, polyesters might be the basis of enzymatically active polymers in place of proteins (polypeptides).

(d) Included here is DNA (as well as protein, polysaccharides, etc.) but in the corresponding box under remote analogues we look for a more general description of information-storing and -replicating molecules.

(e) None of these molecules affords a sufficient criteria for the presence of life, but the accumulation of some of these molecules as components of organisms in high local concentrations is a necessary condition.

(f) An important observation can be made concerning a property of organic compounds in carbon-based living systems that might be extended to all living systems. If, in a living system, there are organic compounds of even moderate complexity, and the production of these compounds involved a template which preserves their steric form, it must follow that organisms exhibit concentrations of compounds containing asymmetric carbon atoms and net optical activity of particular molecular species.

←FIRST PRIORITY SCOPE OF MARS INVESTIGATION→ ←HIGH TEMPERATURE SYSTEMS→
←INTERSTELLAR SPACE→
←JUPITER→

	THE EARTH SYSTEM	(b) CLOSE ANALOGUES WITH CLASS GENERALIZATION	(c) REMOTE ANALOGUES	NON-AQUEOUS	NON-CARBON NON-AQUEOUS	(g) NON-LIQUID
ATOMS	(a) C,N,O,H,P,S, Na,K...				Si,N,B,P...	
(e) MOLECULES IN ENVIRONMENT IN ORGANISMS	H_2O, CO_2, O_2, CH_4, NH_3					
(f) ORGANIC MOLECULES	AMINO ACIDS NUCLEOTIDES METABOLITES					
MACROMOLECULES	(d) DNA, RNA PROTEINS POLY-SACCHARIDES					
CELLS ORGANISMS SOCIETIES	INTELLIGENT COMMUNICATION					

Figure 1

(g) Biochemical criteria for non-carbon, non-aqueous or certainly non-solvated systems of life require a philosophical generalization of biological theory not attempted at this point. The temperature of the habitat is perhaps the single, most important parameter that defines the range of research possibilities of biochemical constructions. Most of our thinking is of course conditioned by the biospheric range of 250-400 K, defined primarily by the properties of water. For our first considerations we have decided to concentrate our efforts on this familiar territory before planning distracting expeditions into even less accessible regions outside this range.

Having constructed such a matrix, the next task is to give probability values to its dimensions and adjust the scales so that equal areas are equally probable. A rational experiment or series of experiments minimizes the effort required to explore this surface.

For this procedure to be useful it is neither necessary nor presently possible for it to be carried out with rigor: the uncertainties of the solutions will, however, become more deductively ascertainable functions of the inprecisions of the stated premises.

The usefulness of a matrix of the chemical and biological properties of living system versus premises of different order of generalities would be enhanced if there also existed an exhaustive categorization of methods of experimental observation. Then one could be sure that one had arrived at a logical choice of experimental objective and at the same time the optimum technique. It is not possible at present to make such an exhaustive

categorization. To be able to do so would imply that there existed a complete theory for chemical behavior. At present there is hardly a complete description of the behavior of large molecules, let alone a theory. It still may be worthwhile to attempt such an enumeration even though it be limited to a specific region of possible experiments. For example, a listing of all the possible ways of detecting hydrogen and its isotopes would demonstrate the enormous range of techniques the exobiologists should keep in mind in arriving at his research decisions. It is obvious that this is a matter of continuing concern. The means of satisfying the parameters such as incident flux and detectivity associated with any technique are constantly changing so that one must continually reexamine the experimental choices made. This also makes it a matter of importance to have a framework into which one can put new data pertinent to a particular experimental approach. That is to say that an exhaustive organization of experimental methods coupled with a system of scientific information storage and retrieval would be a powerful tool in making experimental decisions and might even bring to light completely new methods.

3. Multivator

Multivator represents an attempt to generalize the instrumental requirements of exobiology experiments. We are designing an instrument to meet the constraints of Mariner-type missions and which will be able to carry out a wide range of biochemical experiments. A simple set of performance

MULTIVATOR PARAMETERS

REACTION CHAMBER

VOLUME	: $\approx$ 0.1 ml
NUMBER	: 15; 12 RECEIVE A SOIL SAMPLE OF $\approx$ 5 mg; 3 ARE BLANKS
MODE OF OPERATION	: REAGENTS STORED DRY IN VESSEL; SOIL INSUFFLATED; SOLVENT INJECTED (WATER), INCUBATED; MEASUREMENTS MADE. NOTE THAT SOME REAGENTS MAY BE STORED AS TIME-RELEASE CAPSULES.
TEMPERATURE	: $\approx 30^{\circ}$C (UNCERTAIN OVER WHAT PERIOD OF MARTIAN DIURNAL CYCLE THIS WOULD BE MAINTAINED).

MEASUREMENTS

TYPES	: FLUOROMETRY, COLORIMETRY, NEPHELOMETRY. WAVELENGTH RANGE AVAILABLE 300-600 mμ. LAMP POWER MAY BE ASSUMED TO BE ABOUT 10 MICROWATTS / mμ. DETECTIVITY IS ABOUT 1 PART IN 10^6 OF INPUT LIGHT FOR FLUOROMETRY OR NEPHOLOMETRY AND 1 PART IN 100 FOR COLORIMETRY. DIFFERENT INPUT AND OUTPUT FILTERS MAY BE CHOSEN FOR EACH REACTION CHAMBER.
FREQUENCY	: ONE READING ON ALL CHAMBERS EVERY 15 MIN.
DATA FORM	: EACH READING QUANTITIZED INTO BINARY CODE OF 6 BITS (64 LEVELS). MAY BE LINEAR OR LOG.

Figure 2

characteristics of this instrument becomes the interface the biologists and biochemists in our laboratory have to cope with, not the total complexities of space missions. We hope, in time, to broaden the range of experiments that Multivators can carry out and to design other instruments with similar breadth of purpose.

3.1. Present Multivator parameters

The interface characteristics of the current design are shown in fig. 2. The following possible changes would require only minor modifications: (a) non-aqueous solvents; (b) different solvents in each chamber; (c) measurement of conductivity, pH, and of radioactive reaction products; and (d) increase reading frequency to every five minutes and number of quantitization levels to seven bits (128 levels).

It should be remembered that the system will be in dormant storage for the six-month interval of space flight, and that all components of the experiment must withstand this rest period. Temperature will be maintained above freezing.

According to present sterilization criteria, the components of an experiment must be amenable to heating at 135^{o} C for 24 hours. We have not yet met this requirement for the assays we are investigating.

3.2. Mark I Multivator design

The design and fabrication of a device of the Multivator concept has been undertaken in this laboratory during the report period. Limitation on size, weight, and power consumption as well as operation independent of orientation have led to the configuration of the present model, designated Mark I.

This particular design is based on the use of substrates tagged with a fluorogenic indicator for the detection of enzymes characteristically produced by soil microbes. The design is in accord with the parameters indicated in the proceeding section. It lends itself to the use of radioactive tagging techniques with only minor modification of the existing instrument.

Mark I is designed to analyse a dust sample, collected from the planet surface and delivered to the reaction chambers as an aerosol. A number of sample collection methods are being investigated including vacuum cleaning and electrostatic pick up.

The photographs depict the bench model of the device. The complete Multivator capsule is about 25 cm long, 7 cm in diameter and weighs 380 g.

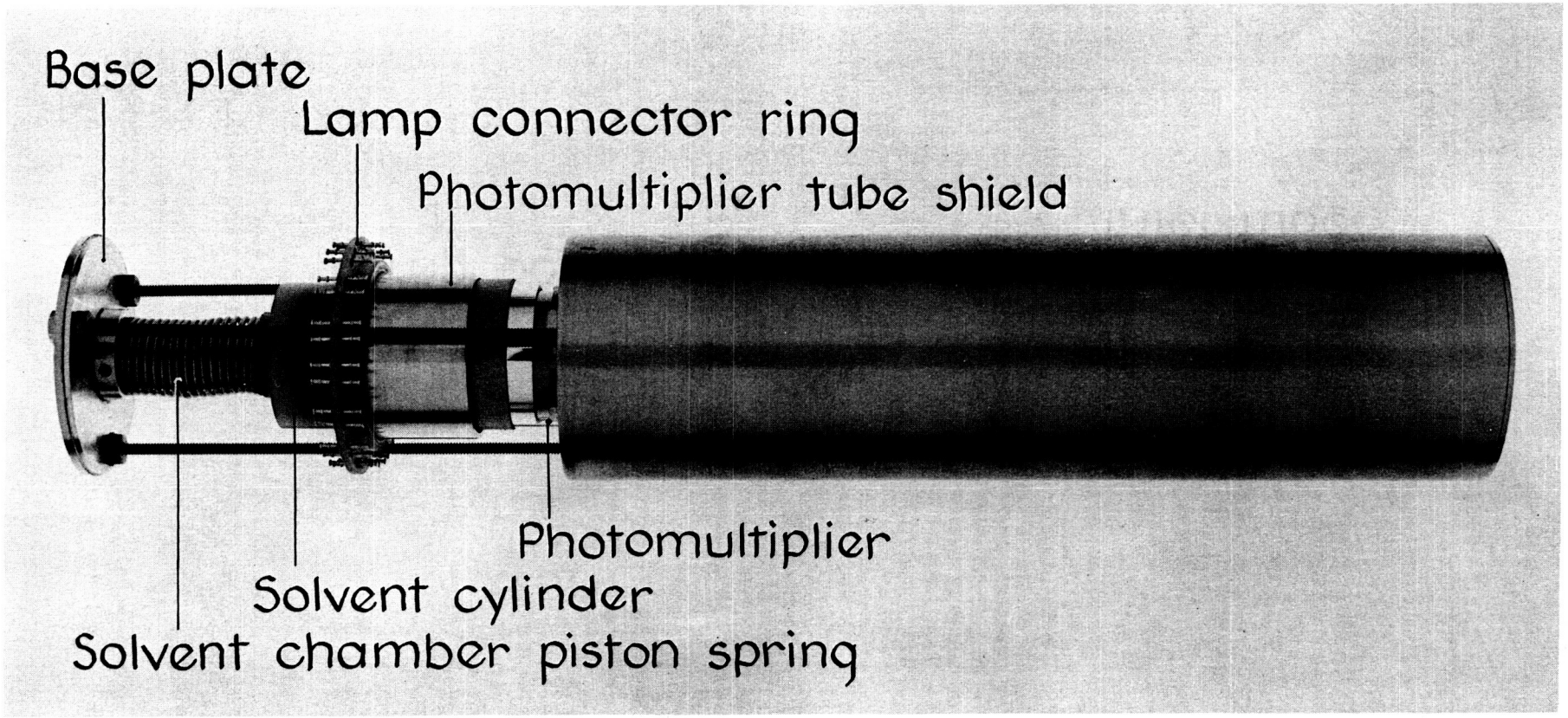

Base plate
Lamp connector ring
Photomultiplier tube shield
Photomultiplier
Solvent cylinder
Solvent chamber piston spring

Figure 3

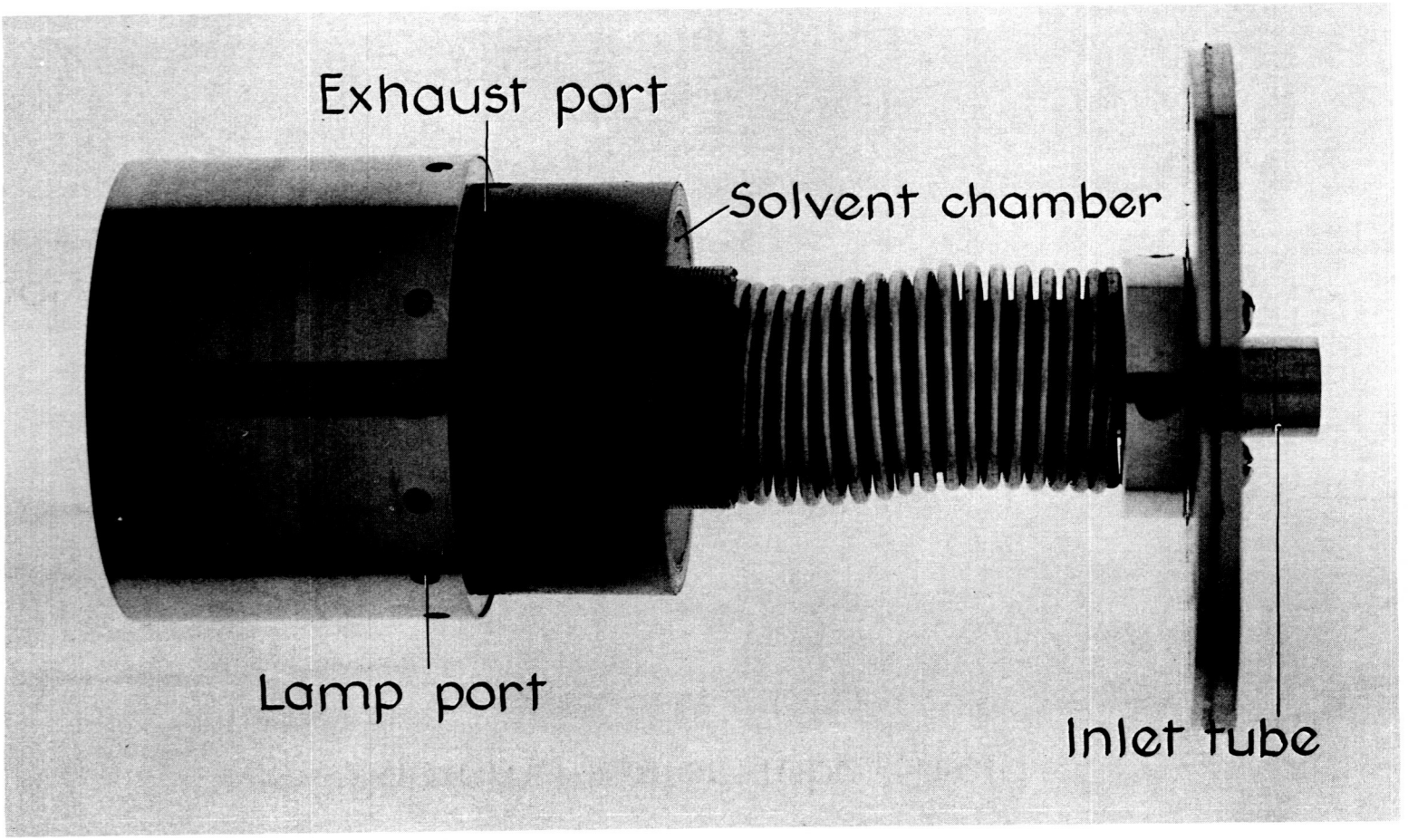


Figure 4

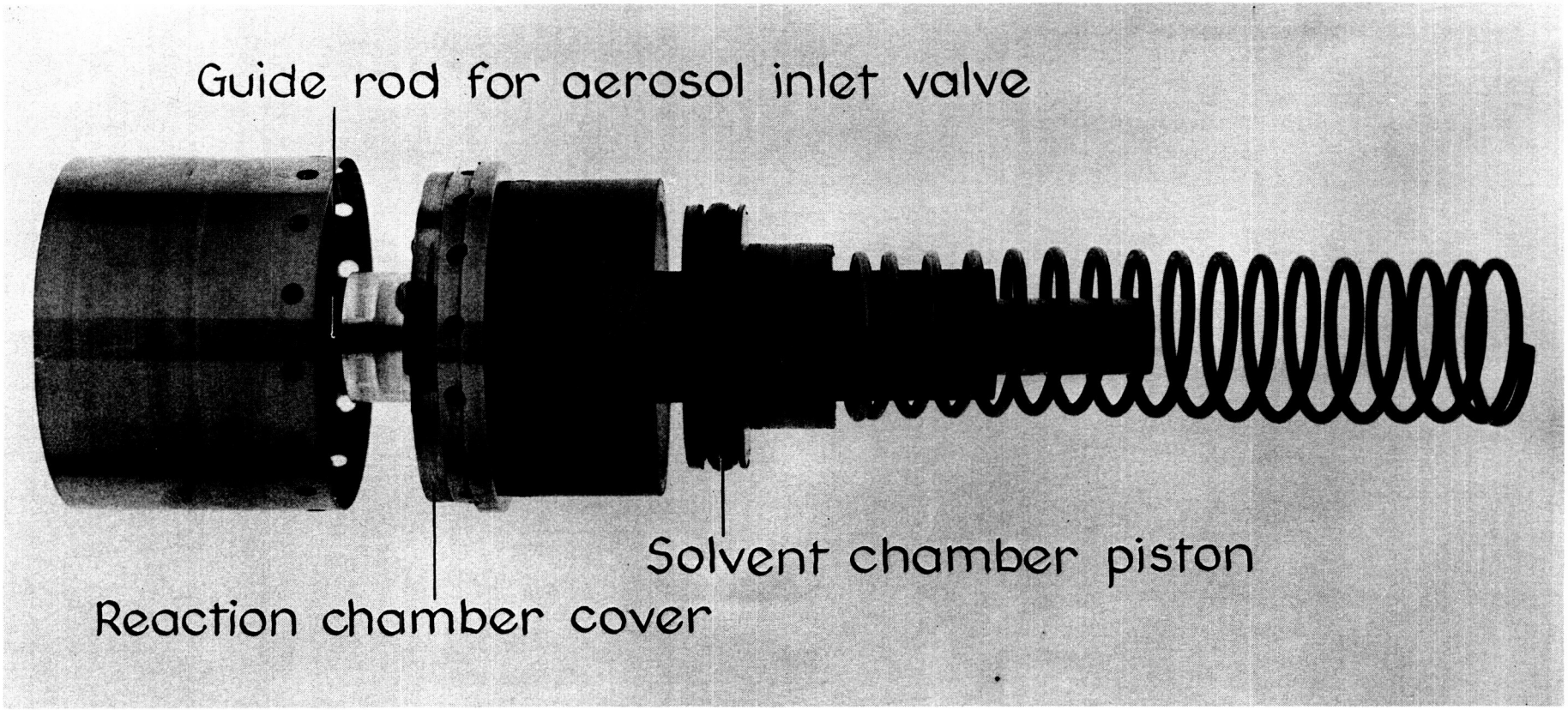

Guide rod for aerosol inlet valve
Solvent chamber piston
Reaction chamber cover

Figure 5

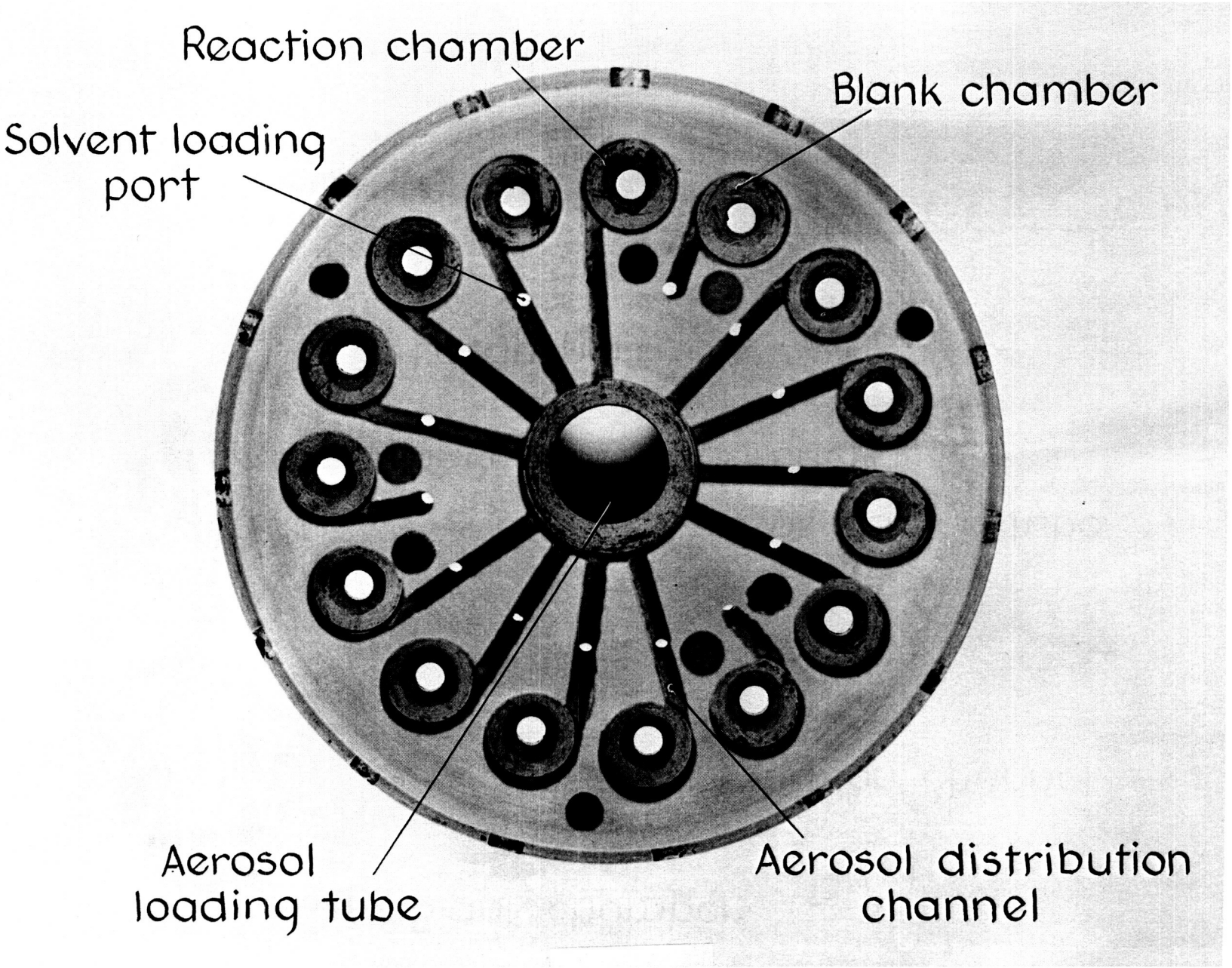

Reaction chamber
Blank chamber
Solvent loading port
Aerosol loading tube
Aerosol distribution channel

Figure 6a

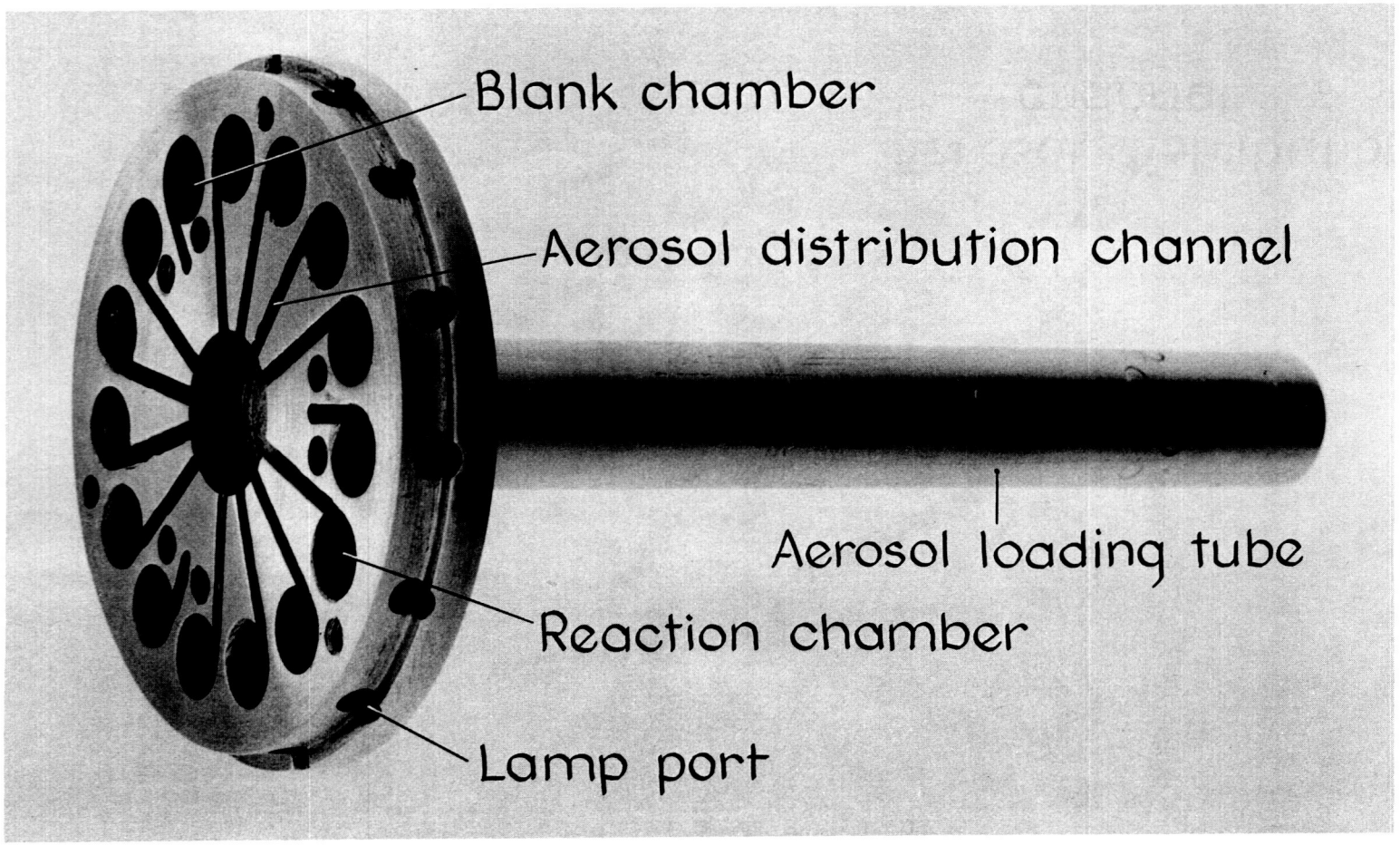

Blank chamber
Aerosol distribution channel
Aerosol loading tube
Reaction chamber
Lamp port

Figure 6b

Fig. 3 shows the device as it is removed from the capsule. From the upper left the components are: base plate, solvent chamber piston spring, solvent chamber, lamp connecter ring, photomultiplier tube shield and photomultiplier.

In fig. 4 the aerosol inlet tube is visible running through the center of the solvent liquid chamber piston spring. The reaction chamber exhaust ports can be seen cut into the lower end of the solvent chamber. The ports in the photomultiplier housing are for the fluorescent exitation lamps, one for each reaction chamber. These lamps will be turned on sequentially to obtain a reading from each reaction chamber.

Fig. 5 affords a view of the solvent chamber piston and the reaction chamber cover. The guide rod for the aerosol inlet tube shut-off valve may be seen protruding from the center of the reaction chamber cover. This valve is spring loaded, fuse-wire actuated and closes after filling is complete.

Fig. 6 shows the individual reaction chambers, the aerosol distribution channels, the solvent loading ports in the channels, the end of the aerosol loading tube and the air exit ports in the bottom of the reaction chamber. This configuration with 15 reaction chambers is designed for three experiments, each with four controls. The reliability and interpretibility of any assay is obviously dependent on adequate controls. As an example of one of the

controls, those chambers which do not have inlet channels to the aerosol inlet tube are designed to test the stability of the fluorescent substrate after the long space voyage.

The operation is as follows. Dust carrying the microbial sample is filtered to ensure no oversize particles and is then blown through the inlet tube and into the reaction chambers. The dust is collected by a sticky coating on the chamber walls, and the dust-free air exhausts out the port in the rear of the chamber. This method of collection has been experimentally shown to collect about 96% of the dust in the aerosol. The aerosol inlet valve then closes, sealing off the individual reaction chamber. A small rotation of the solvent chamber closes off the air exhaust ports and aligns solvent filling ports in the solvent chamber with those in the inlet channel. The solvent chamber piston then injects the solvent into the reaction chambers. The substrates which have been stored dry in the chambers are dissolved and the reaction begins. After a preset reaction time, the fluorescent exitation lamps are then turned on sequentially, and the fluorescent level in each chamber is detected by the photomultiplier tube. This information is then reduced to digital form and transmitted to earth.

3.3. Mark II Multivator

Further refinements of the mechanical design are being carried out by a separate program under the direction of Professor J. Arnold in the Mechanical Design Department. We are working closely with Professor Arnold's group in this endeavor.

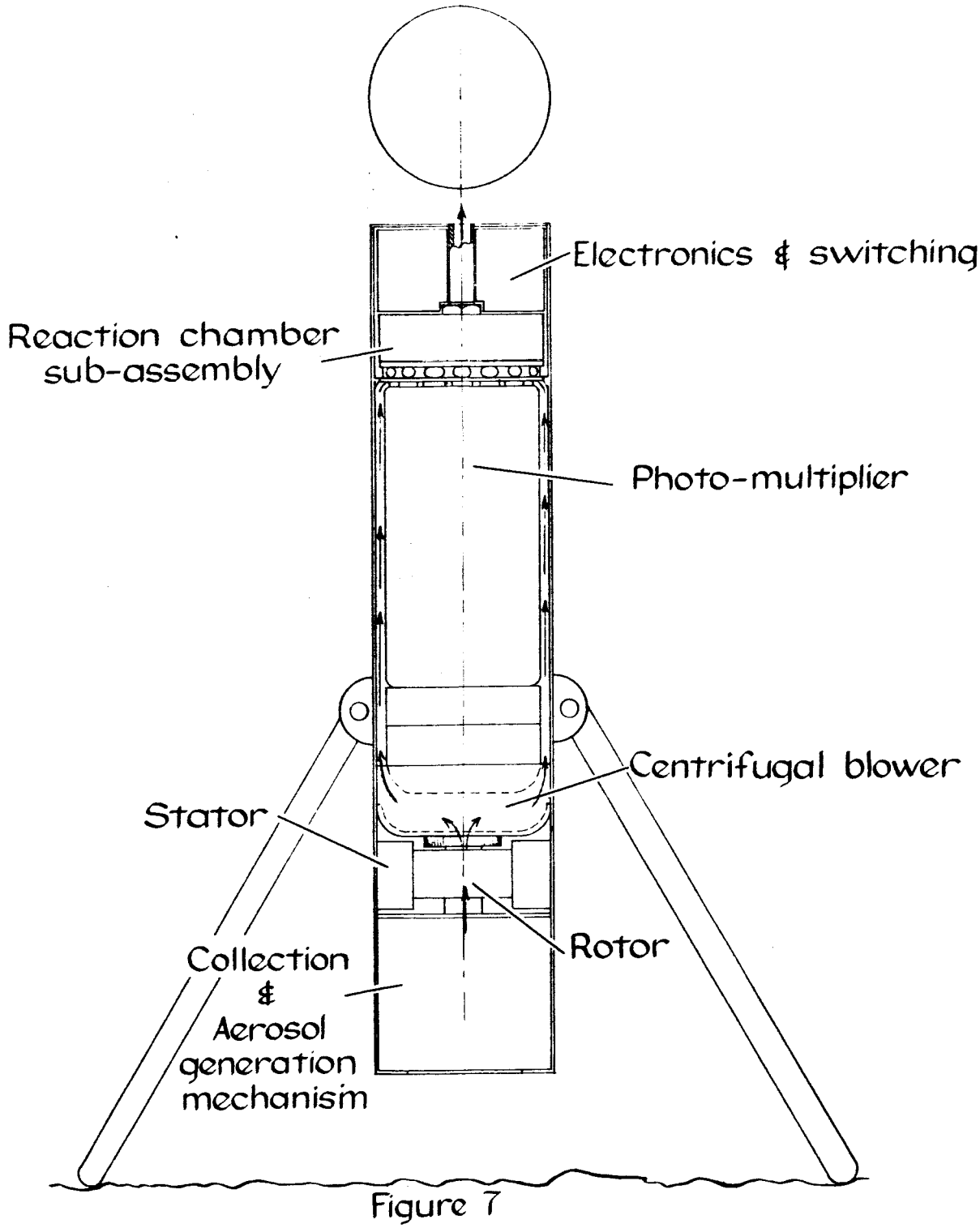

Electronics & switching
Reaction chamber sub-assembly
Photo-multiplier
Centrifugal blower
Stator
Rotor
Collection & Aerosol generation mechanism

Figure 7

Notes~

1. System shown open to aerosol flow stages 1-4 (arrows indicate path of aerosol thru one chamber)

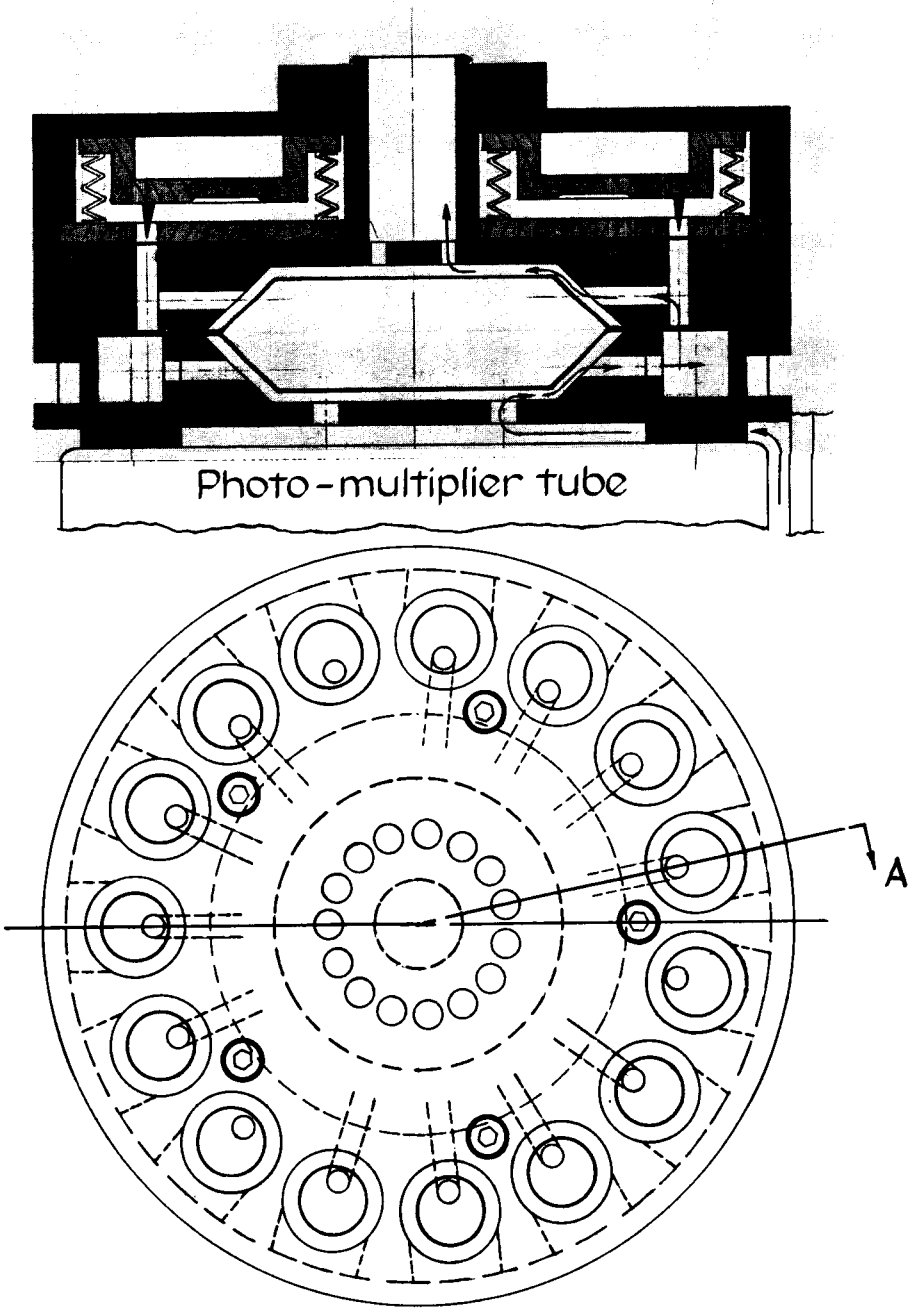
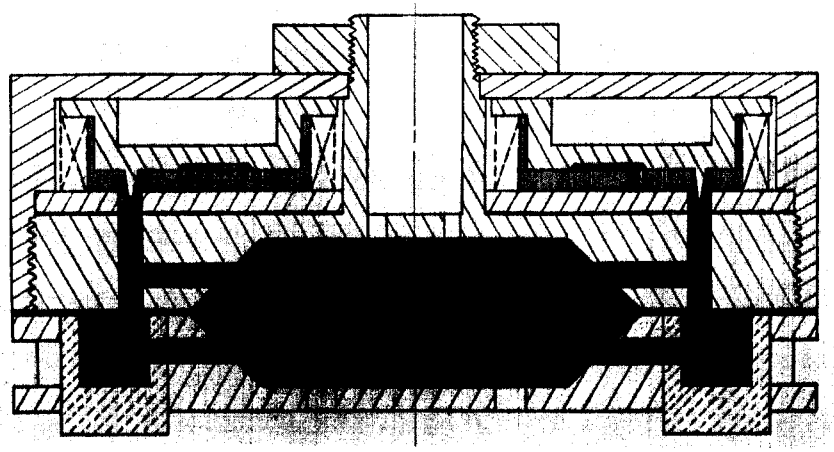
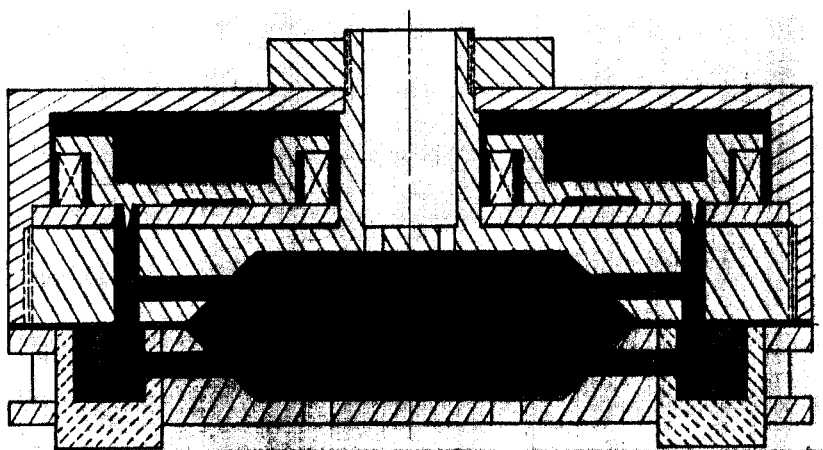


Figure 8



Stage #5
Aerosol passages sealed



Stages #6-8
Aerosol passages sealed~solvent injected

Sequence of events

Stage	Description
1	Eject from capsule
2	Erect on legs
3	Start collection pump
4	Pump air & dust thru chambers
5	Fire valve squib & seal air passages
6	Fire solvent & fill chambers
7	Incubate
8	Turn on lights sequentially & note results

Figure 9

For the sake of completeness we will briefly review the present state of design of the Mark II Multivator and point out some respects in which it differs from Mark I.

The Mark II Multivator is mechanically different but is electrically and optically the same. Seals are replaced by bellows, and explosive charges are used to supply the energy for mechanical operations. The overall size, weight and shape remain about the same. The light source system, photomultiplier detector and assay techniques remain essentially the same. Fig. 7 gives an overall diagramatic view. The Multivator cylinder is ejected from the capsule and erects itself by means of the adjustable legs. The collection system is being designed as a separate element and is not shown. Dust from this system is blown into the test chambers. The chambers and the aerosol flow path is shown in fig. 8. The firing of a squib charge seals the air passage as shown in the upper part of fig. 9. A second squib is fired resulting in a piercing of membrane containing the solvent in its chambers and the release of solvent to the assay chambers. Bottoming of the solvent chamber piston seals the fluid injection ports. This is illustrated in the lower part of fig. 9. The reactions and measurements then proceed as in the previous model. The Multivator is attached to the capsule which contains the telemetry equipment.

3.4. Mark III Multivator

We have set as a goal the ability to measure abundances as low as 100 to 1,000 bacteria in samples of 1 to 10 mg of Martian soil. We have not yet reached this goal with the present instrument. The limitations do not seem

at present to be the sensitivity of the fluorescent assay itself but rather the chemical noise introduced by the rate of hydrolysis of the substrate. We need to find methods of improving this stability or of finding substrates with similar fluorescent cross sections and improved stability. We are now working on a Multivator operating in a manner which gets around these difficulties. To the extent that the chemical noise is distributed throughout the volume of the sample being observed, while the fluorescent signal of biological origin is not, we can improve the sensitivity in proportion to the reduction of the observed volume. We are thus taking advantage of the fact that the great majority of soil bacteria grow in colonies which are rather firmly bound to the soil particles, giving much higher local metabolic activity in the vicinity of a particular soil particle than the average activity of a complete sample.

With the foregoing thought in mind, the following system is being considered. The soil particles are to be more or less evenly distributed over the surface of a substance, such as a gel, which contains the fluorescent substrate. If the diffusion rate of the product is low compared to the rate of enzymatic activity, there will then be found concentrations of fluorescent product in the vicinity of bacterial colonies. This local concentration may then be detected above the background by a suitable scanning technique. If these local concentrations occupy a volume equal to or less than the resolution of the system, the improvement of signal over background is in the ratio of total enzymatically active points to total points scanned. It should be possible to make this improvement 10^3 or better.

As any device which is to be sent on a mission must of necessity be power-limited, the best possible instrumental signal-to-noise ratio for fixed power input must be determined. It can be shown that for a given light source flux, the signal-to-noise ratio becomes dependent only on the absolute noise of the detector, regardless of any other considerations. This immediately indicates that the flying spot scanning system with the low-noise photomultiplier detector is an appropriate choice. A laboratory flying spot scanning system has been built to test the proposed techniques. Preliminary experiments with the scanner have been encouraging. With this laboratory instrument, further studies of this technique will be pursued. If they are successful, design parameters of a Mark III Multivator will be determined and a model constructed. In addition we will consider what other new dimensions this adds to the experimental possibilities of Multivator.

4. Multivator assays

The assays we are investigating fall into two general categories. The first detects the presence of hydrolytic enzymes by fluorometry, testing for the catalysis of $RX + H_2O \longrightarrow RH + XOH$. The bases of the test is the release of XOH which differs from XR in being highly colored, or, better, fluorescent. For example, R = phosphate; X = alpha naphthol to constitute a fluorometric assay of phosphatase. The second uses the techniques of membrane separation to measure the production of molecules with membrane transmission properties differing widely from those of the substrate.

The specific permeability of membranes could thus furnish a simple approach to biochemical analysis especially suited to the constraints of exobiological experiments. At present we have been principally concerned with the separation of gaseous or monomeric reaction products released from larger molecules under the action of specific enzymes or of whole organisms. The identification of the end-product molecules could be dependent on the use of radioactive isotopes, for example, the detection of metabolic CO_2 from C^{14} labelled nutrient substrates. Fluorometry, however, could still be the detection mechanism if the polymer substrate were labelled with a fluorescent molecule. Our biochemical studies have been directed mainly towards evaluation of the phosphatase assay and achieving an understanding of membrane permeability which would permit exploitation of membrane operation techniques. Surprisingly little previous work has been done on the permeance of membranes on a wide enough dynamic range to be pertinent to the present applications.

Phosphatase activity has been given particular attention for the following reasons:

(a) It is widespread among terrestrial organisms.

(b) It catalyses a wide range of reactions with moderate specificity.

(c) It is involved with the unique role of phosphorus in metabolism and energy transfer, which may very well be a universal characteristic of carbon-based aqueous living systems.

(d) It is capable of being detected with relatively high sensitivity.

4.1 Hydrolytic Assays

The occurence of phosphatase activity in terrestrial soils has been the object of a systematic study. The assay technique used was the fluorescent product formation which will be discussed shortly. A wide variety of soils from major climatic and geological regions, including samples from the Mojave Desert sands and Death Valley salt flats were screened. These soils were obtained from a variety of sources, including the Soil Survey Laboratory of the University of California, Berkeley. Some uncertainty exists about the effect of soil handling after collection, and no data is available on this point. All samples were air-dried prior to their assay. Without a single exception, all of the fifty-nine soil samples tested were found to possess measurable phosphatase activity. Of these, fifty-one (or 86%) exhibited phosphatase activity when assayed at pH 7.6, while 95% showed activity at pH 5.6. No correlation was found between the apparent pH of the soil, and the nature of its phosphatase activity. All of these activities could be destroyed by heating, or inhibited by certain chemicals, such as mercuric chloride. In the case of mercuric chloride inhibition, a reversal could be obtained by adding a sulfide. These observations represent strong evidence for the biological nature of this phosphatase activity in soils, and for its ubiquity.

The source of these activities, however, is a different matter. Most soil bacteria are tightly bound to soil particles and no reliable viable count by a direct or indirect method could be made. Little is known about the synergistic relationship and nutritional requirements of these organisms, and a wide and deep area of investigation is open in this field. We are unable to determine at this juncture whether the phosphatase activity of soils represents only a current bacterial population, or whether it includes

a substantial storage and preservation of active proteins from previous generations of organisms as well. There is good evidence to suggest that clay montmorillonites are able to absorb, stabilize and protect proteins.[2]

The reference soil, collected in the vicinity of the Stanford University Medical Center, has been used over a period of eighteen months. During that time the phosphatase activity of the sample, stored in a closed jar at room temperature, remained constant after a small initial decrease. Thus, it is not unreasonable to hope that extraterrestrial surfaces might also be able to preserve biological activity representing some of the past history of the soil.

The synthetic substrate used for these assays was alpha-naphthyl phosphate,** (I) which is commercially available. Alpha-naphthyl phosphate does not fluoresce, but alkaline solutions of alpha-naphthol exhibit a marked fluorescence peak at 460 mμ, when activated with light at 336 mμ. Because of the short activating wave-length, there often was a considerable fluorescence background due to soil components. Quenching and stability problems, in addition to marked physical adsorption of alpha-naphthol by alkaline soils in particular, were overcome by appropriate controls in the laboratory but would become considerably more serious in extraterrestrial investigations. In an attempt to improve the signal-to-noise ratio for these assays, samples of fluorescein** (II) and fluorescein diphosphate** (VI) were obtained from Dr. B. Rotman and Dr. J. Zderic of the Syntex Institute for Molecular Biology. These substances were exhaustively purified by paper chromatography prior to use. In the phosphate ester form, both of these substrates exhibited low fluorescence, but their hydrolysis products were strongly fluorescent at 520 mμ, when excited at 480 mμ. The

** See Glossary of Organic Substances

relative stability of the three substrates compared as follows:

		pH 6 (Tris buffer)*		pH 8	
		4°	24°	4°	24°
Alpha-naphthyl phosphate**	(I)	.001	.007	Not detectable	.003
Fluorescein monomethyl ether monophosphate**	(VII)	.08	.25	.08	.33
Fluorescein diphosphate**	(VI)	.001	.014	.010	.025

* Data obtained by fluorescence measurements, expressed in per cent hydrolysis of substrate per hour.

It can be seen that alpha-naphthyl phosphate is the most stable of the three, and that fluorescein monomethyl ether monophosphate** (VII) is very unstable under the assay conditions used. The background fluorescence produced by these substrates corresponds to a hydrolysis rate produced in an hour, by the phosphatase activity of approximately 10^5, 10^6 and 10^7 E. coli bacteria respectively. The alpha-naphthyl phosphate value does not include the soil absorption factor nor the frequent native soil fluorescence, which tended to significantly decrease the signal-to-noise ratio. Both the fluorescein monomethyl ether monophosphate** (VII) and the fluorescein diphosphate** (VI) were found to be quite unstable and had substantial fluorescence as the phosphate esters, perhaps due to slow hydrolysis even in the solid form. In any case, while these substrate have a very high inherent sensitivity to phosphatase activity, the assay thresholds correspond to many more bacteria than we have any right to expect to find in 100 mg. samples of

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** See Glossary of Organic Substances

extraterrestrial soils.

The results of this investigation are presented in a more complete form in a report prepared by Dr. L. Hochstein included as appendix A. A number of approaches are being examined in an effort to circumvent, if not to solve, these problems. Among these are attempts to increase fluorescence yields, to localize the substrate on a gel in order to detect local spots of intense fluorescence where soil particles have been deposited, then to activate and scan these areas with the flying-spot device mentioned earlier.

A number of other fluorescent substrates are being screened, but fluorescein still seems to be the most attractive possibility, if only one could overcome the unstability of its phosphate esters.

There is a great deal of confusion in the literature on the compound fluorescein. Actually two distinct compounds exist and together they represent a redox pair. They are fluorescin** (V) and fluorescein** (II). Given below is a glossary of organic substances discussed in the text. The roman numerals in parenthesis following the common chemical names refer to compounds described in the glossary.

** See Glossary of Organic Substances

The oxidized substance in alkali is uranine (III), the structure which is the actual fluorescent hybrid. The phosphate esters of dihydrofluorescein (IV) are among the compounds we are investigating.

4.2 Membrane Separation

More than a century ago, Thomas Graham made use of the potentially powerful separatory tool, the semipermeable membrane, in a device called a dialyzer to separate low molecular weight substances from colloids.[3] Subsequent workers have succeeded in refining the techniques to a state where L.C. Craig recently reported data suggesting that separation of the epimers glucose and galactose (as well as sucrose and lactose) is feasible by dialysis through treated cellophane.[4] It is in this latter context that a modification to Multivator where a semipermeable membrane is employed to achieve a separation of the smaller enzymatic breakdown products from the substrate seems reasonable. One potential use of the modified Multivator would be to house an assay similar to the one porposed by G.V. Levin.[5] For this assay a semipermeable membrane would be selected to separate the carbon-14 labelled product, i.e., CO_2, from the labelled substrate. This is only a particular example of a wide range of assays that could be carried out by membrane separation techniques. In addition to the discussion below a more complete description of this work is given in a report by J. Lundstrom included as appendix B.

To illustrate the magnitude of separation involved in such a life-detection device, one can consider as a model that the substrate broth contains carbon-14 labelled glucose in 10^{-3} molar concentration. The theoretical limiting specific activity per carbon atom is 65 mc/mg-atom (Appendix B, sec. 5.1) so that assuming all of the carbon in glucose is labelled[6], then the specific activity of the CO_2 produced is 65 mc/mmoles. If the limiting sensitivity of the detector is set at 10^2 dpm, then the minimum number of molecules of CO_2 detectable is 4.2×10^{11} molecules (Appendix B, sec 5.2). If the assumption is made that the time needed for most of the CO_2 molecules, e.g., 90% produced by the breakdown of substrate to diffuse through the semipermeable membrane into the detection chamber is short in comparison to the time for the production of CO_2, then an estimate of the CO_2 concentration in the 1 ml reaction chamber is 8×10^{-10} molar. The corresponding ratio of the concentration of glucose to CO_2 is about 10^6:1.

Using CO_2 production data for Escherichia Coli given by Clifton[7], the time needed for one bacterium to produce 4.7×10^{11} molecules of CO_2 is 2×10^2 hours (Appendix B, sec. 5.3). The 2×10^2 hours assumes that the single bacterium has not multiplied during this period; however, the more likely case is that the bacterium is not only able to survive in the chamber but will also multiply. For an optimistic generation time of 30 minutes, the time needed to produce 4.7×10^{11} molecules of CO_2 is reduced to about 4 hours; for a generation time of 2 hours one might expect to wait 16 hours for the detectable amount of CO_2 to be produced.

The above considerations allow one to state the demands placed on the membrane for separation in the life-detection device. The membrane must retard the flow of glucose into the detection chamber so that less than 7×10^{10} molecules of glucose diffuse into the 1 ml. detection chamber during the first 20 hours of the experiment (Appendix B, sec 5.4). Twenty hours was chosen on the basis of the 16-hour production time for CO_2 with 4 hours taken as a sufficient measuring period. The same membrane must allow 90% of the CO_2 produced in trace concentrations of 8×10^{-10} molar in the reaction chamber to diffuse into the detection chamber within a period of time that is short compared to the production time for CO_2. If the production time is taken as 16 hours, then the time for 90% of the CO_2 to diffuse through the membrane should be about 1% of that production time or about 10 minutes. A concentration difference across the membrane for CO_2 can be provided by coating the scintillator beads in the detection chamber with an anion-exchange resin that will trap the CO_2 diffusing through the membrane and keep the concentration of the free CO_2 in the detection chamber low with respect to the reaction chamber concentration. The proposed separation seems even more remarkable when one considers that the molecular weights (a crude approximation to molecular size) of glucose and CO_2 differ only by about a factor of four. Clearly, one needs to scrutinize the process of membrane permeability to effect the proposed separation.

A typical permeability curve where one plots the number of moles of solute that have passed from the upstream chamber of high solute concentration through the membrane into the downstream chamber of low solute concentration versus time is shown in fig. 10. One finds three distinct regions on the

curve, i.e., regions of increasing slope, constant slope, and decreasing slope.

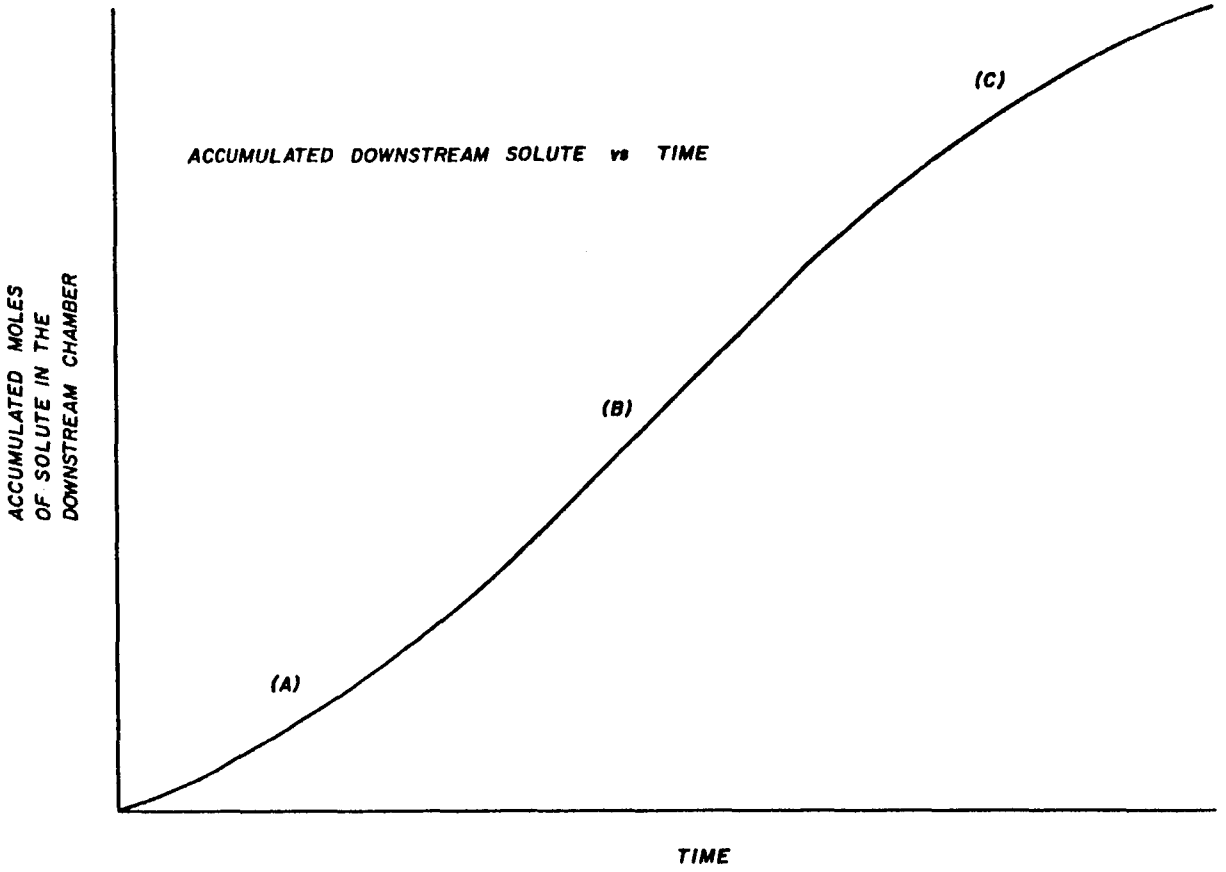

Fig. 10

In region (A) the membrane is not saturated with the solute yet, and the resulting apparent permeability coefficient is smaller than the true permeability coefficient. In this region the apparent permeability coefficient is increasing with time. In region (B) the membrane is saturated with the solute so that there is a steady-state of solute flow across the membrane. Region (B) is easily recognized by its constant slope. The true permeability coefficient can be calculated from this constant slope. Region (C) is

typified by a decreasing slope of the permeability curve with time. This decreasing slope is a result of a decreasing concentration difference across the membrane. Several investigators, e.g., L.C. Craig[4] and E.F. Leonard[8], have used region (C) to calculate the permeability coefficients of fast membrane-solute systems, e.g., diffusion of sugars and low molecular weight electrolytes through cellophane, by measuring the decrease in the upstream chamber solute concentration as the upstream chamber is depleted with time.

Since the proposed glucose-CO_2 separation is only necessary over some finite time period, one could maximize the separation by prolonging the time needed for the membrane to become saturated with glucose, i.e., the time needed to reach region (B), and thereby keep the glucose permeability at some value less than its true permeability. The same membrane should be readily saturated with CO_2 so that the CO_2 permeability is maximized over the 20-hour period.

Our approach to this separation problem has been directed in part at gaining some fundamental information about the factors affecting the time needed for steady-state flow of the solute across the membrane. Studies with two fluorescent dyes, Na_2Fl (disodium fluorescein) and Na_2E (disodium eosin), and swelled cellophane (presoaked in H_2O for a period greater than one day prior to its use in an experiment) indicate that the upstream solute concentration and the membrane thickness affect the time for steady-state by some non-linear relationships. These relationships are discussed more fully in appendix B.

Some published data suggests that the time for steady-state is also related to the temperature, i.e., the time for steady-state decreases as the temperature increases.[9] A fourth factor affecting the time for steady-state, the permeability coefficient itself, is suggested by some incomplete data from the dye-cellophane permeability study. The intuitive relationship here is that the time for steady-state is inversely proportional to the permeability coefficient.

The dependence of the time for steady-state on the upstream chamber solute concentration is a matter of some concern since the CO_2 concentration is anticipated to be extremely small. Some permeability data of CO_2 through rubber published by J. Dewar[10] show that the permeability coefficient increases at high pressure (in the range of 10 to 20 atmospheres). However, we have not succeeded in finding permeability data for low pressures (or concentrations) of CO_2 that give convincing evidence either for or against continuation of the above relationship between the permeability coefficient (and the time for steady-state) and the pressure of CO_2.

The permeabilities of Na_2Fl through three hydrophobic membranes chosen from the published literature as having high permeability coefficients for CO_2 have been measured. Pilot studies were started on the permeation of sodium fluorescein to take advantage of the sensitive fluorometric assay system already set up here. Fluorescein would simulate organic acids of modest molecular weight. The comparability of permeation behavior to non-polar solutes

is unknown; nevertheless calculations will be presented as if glucose were comparable to fluorescein. The measurement of Na_2Fl permeability through the three hydrophobic membranes and the isolation and preliminary investigation of the factors affecting the time for steady-state sum up the present work of the dye permeability studies.

Actual permeability measurements of radioactive-labelled glucose as well as other organic metabolites and CO_2 are planned for the near future. Particularly important experiments with CO_2 will be the effect of upstream chamber concentration on the time for steady-state and the effect of pH on the permeability coefficient. The effect of pH on the concentration of free CO_2 is anticipated to be an important factor to control in the life-detection device since the permeabilities of the bicarbonate and carbonate ions have been suggested as being negligible compared to free CO_2.[11] The effect of the upstream chamber concentration of glucose on the time for steady-state will be important to study in order to minimize the leakage of glucose into the detection chamber of the life-detection device.

The necessary permeability cells for studying membrane-solute systems differing in permeability coefficients over a 10^{11} range have been designed and machined out of stainless steel for the radioactivity experiments. The 10^{11} range was arrived at by choosing \$500 as the maximum cost per run for the glucose and 10^5 seconds as the time to obtain an experimental datum point. By relaxing either

or both of these bounds the range can easily be expanded. These three cells are presently supplementing the arsenal of twelve cells used to study the permeability of dyes.

Standard scintillation counting techniques of non-aqueous solutions are planned for the preliminary radioactive assays of CO_2 and glucose in the forthcoming permeability studies of the carbon-14 labelled compounds. The hyamine method for trapping the CO_2 in toluene worked out by Passmann[12] and modified by Eisenberg[13] appears suitable for the CO_2 analysis. Coupling the hyamine method with Van Slyke's[14] wet combustion of carbohydrates seems to be a straightforward technique for the glucose assay. An alternate procedure for the glucose assay would be to reduce the volume of the aqueous glucose solution to less than one ml. by evaporation of the water and then to dissolve the remaining solution in toluene, using dioxane to effect the dissolution[15], or in 1,2 dimethoxyethane[16]. Eventually a technique for counting the aqueous solutions of the CO_2 and the glucose will be devised, since the direct assay is desirable for the over-all convenience in conducting the permeability experiments and for the eventual application in the life-detection device. Use of solid state scintillators, e.g., the anion-exchange resin coated scintillator beads mentioned earlier or finely-divided anthracene[17], seems a promising method for direct counting of aqueous solutions at present.

The fluorescence assay for the dye experiments has employed a Turner fluorometer[18] with sensitivities of 10^{-9} molar for Na_2Fl and 10^{-8} molar for Na_2E.

5. Video Scanning Technique

5.1 Introduction

For the past year we have been carrying out research to investigate the application of video tracking or more generally video scanning techniques to biological problems. This work is closely related to work this laboratory is doing under an Air Force study program (Contract AF 18(600)-1911). This report covers the total effort.

Our initial effort has been, naturally, instrumental. We desired to have a flexible video scanning system that would permit quantification of most of the important variables. That phase has largely been completed and is reported in the section 5.2.

Towards the end of this year we have started to assess the application of the instrument to problems of biological interest. We have chosen as an initial problem the use of the video scanning instrument as a scanning densitometer. This has been chosen not only because it is a useful application but also because it gives a good way of evaluating the instrumentation particularly with regard to analytical accuracy. It also simulates the more complex use as a scanning spectrophotometer. Some efforts have also just started in connection with other applications.

Information analysis of photographs has been initiated using Philco's IMITAC equipment.

5.2 Television Equipment

The camera control circuits and video circuits have been expanded and improved in order to provide better performance, additional modes of operation, and greater flexibility. A block diagram of the system is shown in fig. 11. Fig. 12 is an alternate video system, used for densitometry. This equipment permits differencing, either linearly or logarithimically, on a line-to-line or frame-to-frame basis, over a wide range of frame rates, numbers of lines, and scanning patterns; as well as log-difference operation on a line-integral basis.

The camera control equipment permits: deflection of the vidicon beam over a wide range of rates, for experimental determination of speed-sensitivity trade-offs; various raster patterns for different purposes; and different blanking (storage) times, for investigation of the storage properties of various image tubes. The digitized camera control drives the vidicon deflection coils via improved direct-coupled current-feedback operational amplifers. Low-inductance deflection coils are driven by high-current output stages, permitting traversal of the raster by the beam in either axis direction in times from less than 5us to infinity. Fail-safe operation is provided via deflection-dependent unblanking.

Counters, logic, and digital-to-analog converters provide a choice of four raster formats, and various blanking (storage) times. The number of lines may be any power of 2 from 1 to 12: i.e., between 2 and 4,096. The horizontal sweep may be generated either by the integrate-and-trigger method, with vernier rate control via input current control and step rate

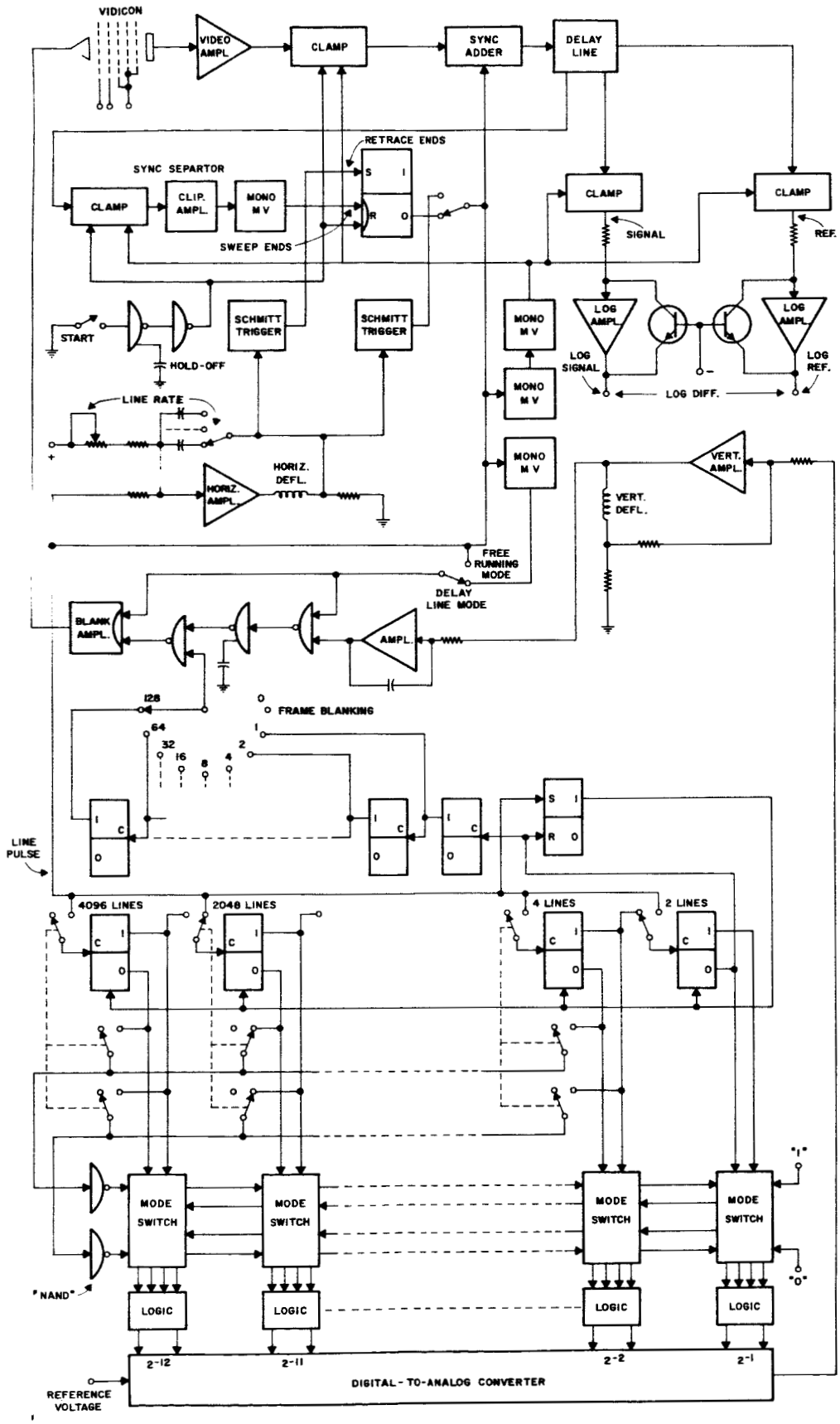

FIG. 11 BLOCK DIAGRAM

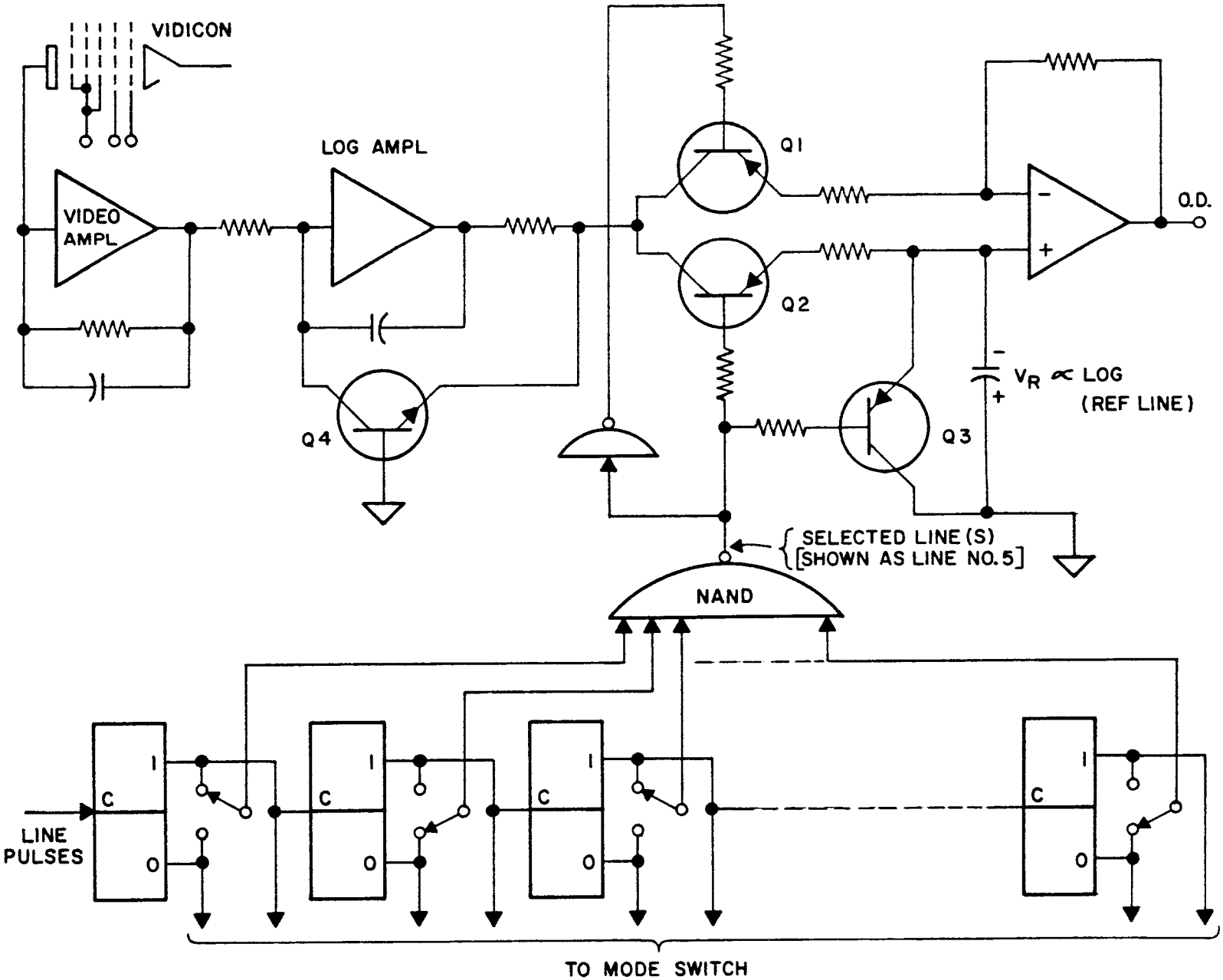

FIG. 12. ALTERNATE VIDEO SYSTEM

control via switching of integrating capacitors, which permits rates from standard TV speed down to arbitrarily slow; or by means of a delay line, which gives rates from standard TV speed down to ½ of that rate. Extension of the counter chain permits the image tube to be blanked frame-symmetrically, with the blanked and unblanked times any power of 2 from 0 to 8; i.e., alternate frames may be blanked, or 2 frames blanked and 2 frames scanned---up to 256 frames blanked and 256 frames scanned. This feature permits investigation of the storage properties of vidicons (special and otherwise) by comparison of the charge read off the target during the first frame (or several frames) after a period of accumulation of photo-induced target charge (with the beam cut off) to the charge read off the target during a sequence of normal scannings (with one-frame accumulation).

The rasters are attained by counting the horizontal pulses (at line rate), and driving a binary digital-to-analog converter with the contents of the counter. The converter, in turn, drives the vertical-deflection amplifier. The number of lines is determined by where along the counter chain the horizontal pulses are fed in. If all 12 counter stages are used 4,096 lines result; if the input is fed to the fourth stage from the end, 16 lines result, etc.

A digital line selector provides a pulse of length 2^n lines (n=0 for 1 line, n=2 for 4 lines, for instance) at any vertical location in the raster, thus permitting monoscope monitor operation, and providing a switch-drive signal for line-integral log-difference operation.

A blanking signal for storage properties studies is obtained by feeding an auxiliary 8-stage counter with the reset signal of the main (line) counter. Thus, the 8-stage counter counts frames. By selecting one stage's output and ORing it with the normal blanking signal, the image tube may be alternately scanned and blanked for various binary numbers (1,2,4,---) of complete frames. See fig. 13e and 13f. This blanking mode of operation may be used with any of the raster formats, numbers of lines, line-generation modes, line rates, etc.

The raster format is determined by the logic which is connected between each stage of the counter and the corresponding bit of the digital-to-analog converter. A conventional staircase raster (fig. 13 d) results from simply connecting each counter stage to the corresponding converter switch.

To get the oddly displaced staircase (fig. 13c) in which each odd count is displaced by half of full scale, all bits of the converter, except the large bit, are driven directly from the counter, as before; but the large bit of the converter is driven by the small bit of the counter. Hence, the converter's output jumps up or down by half of full scale ($\overset{+}{-}$ 1 count) every line. Note that there are only half as many lines as before. This is because, at the converter, it is impossible to have the large and small bits different: half of the count combinations are removed, and the converter cycles twice for each counter cycle.

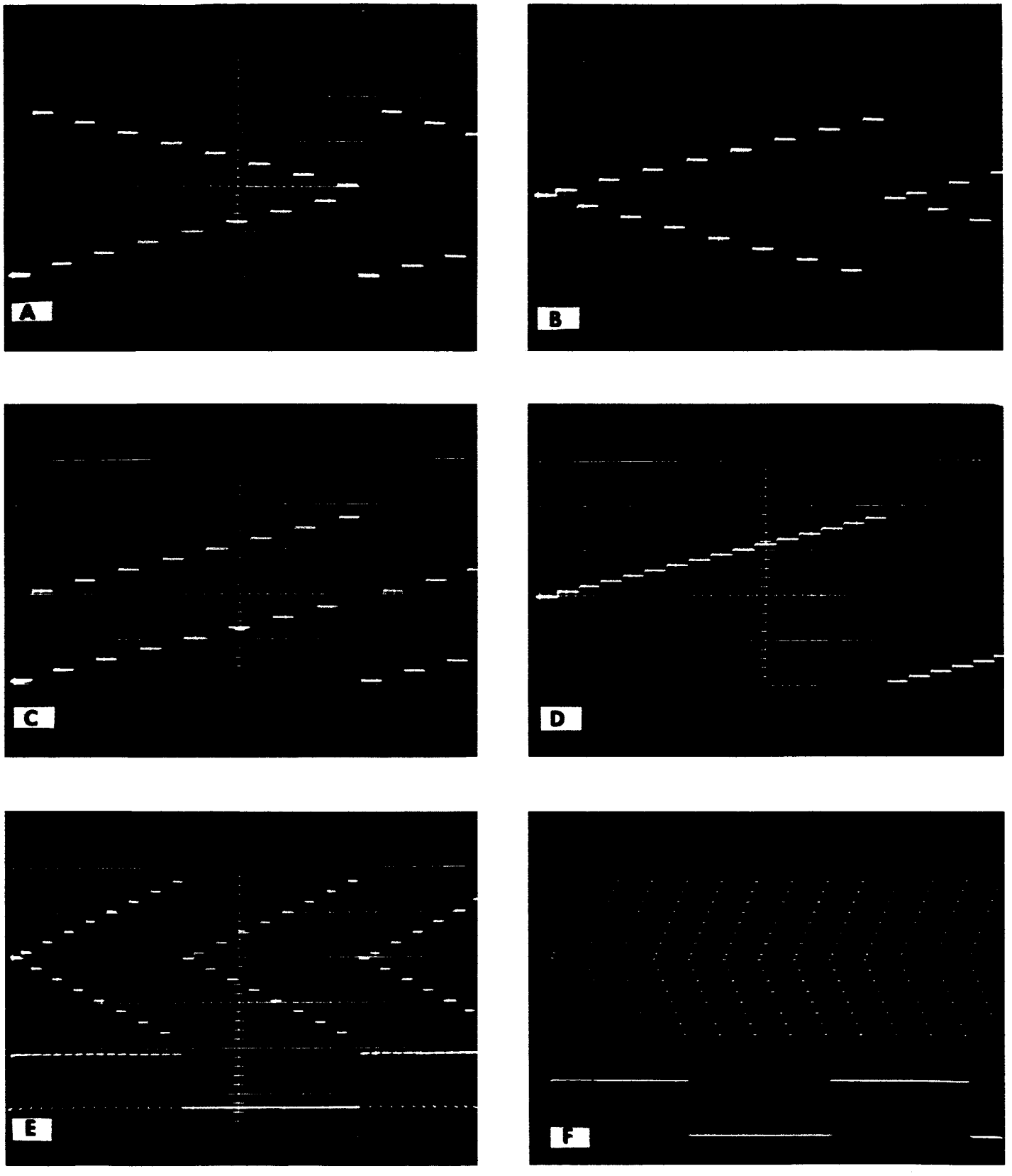

Figure 13

The symmetrically converging raster of fig. 13 a is attained by driving the large bit of the digital-to-analog converter from the small bit of the counter, and driving all other converter bits with the "exclusive or" (non-identity function) of the corresponding bit of the counter and the small bit of the counter. Again, there are only half as many lines as counts, but for a different reason: the "exclusive or" of the small bit with the small bit is always false, so the small bit never changes in the converter and half the count combinations are removed. This does not result in each vertical deflection position lasting for two line times, as one might expect. Since the small counter bit may change all except itself, a change occurs for each line.

The symmetrically diverging raster of fig. 13 b results from driving the large bit of the converter from the small bit of the counter, and driving all other converter bits with identity function (negation of the "exclusive or") of the corresponding counter bit and the small counter bit. Again, the number of vertical deflection positions is half the number of counts, for the logically inverse reason as in the preceding case.

With delay-line sweep generation, video may be superimposed upon the synch in the delay line, thus giving one-line synchronized video delay. Line times of 63us to 250us may be attained with the present delay line. Operation in the delay-line mode has been improved by making the synch pulse of opposite polarity to the video signal, using polarity-sensitive synch separation (which, in turn, allows much smaller synch pulses and

reduced interference with the video signal), and improving the clipping and clamping circuits.

Equipment to operate the vidicon(electrode-voltage supplies, controls, etc.) has been built, to replace less flexible commerical equipment. Changes in supplies, controls, and blanking have been made, in order to operate the vidicon target at ground potential, permitting the use of direct-coupled video amplification. A direct-coupled, bootstrapped, video amplifier utilizing a Field Effect Transistor is used. Such an amplifier eliminates the video droop problem at slow line rates, simplifies clamping, reduces or eliminates the need for post-emphasis, and improves signal-to-noise ratio.

For use as a densitometer in conjunction with the Spinco Model E1 Ultracentrifuge, the video circuits shown in fig. 12 will be used. This system has been successfully tried, using Tektronix Type O operational amplifiers. In this mode a standard (non-interlaced) raster is used, with the lines in the tangential (uniform) direction of the cell image. The video amplifier is direct-coupled, in order to permit very slow scans to be tried, and its response time constant (determined by its feedback network) is a fraction of the line time, so that the output reaches essentially its steady-state value by the end of a line. The video amplifier feeds a logging amplifier, which uses a transistor as the logarithmic element[19] This logging circuit has very accurate logarithmic response over much more

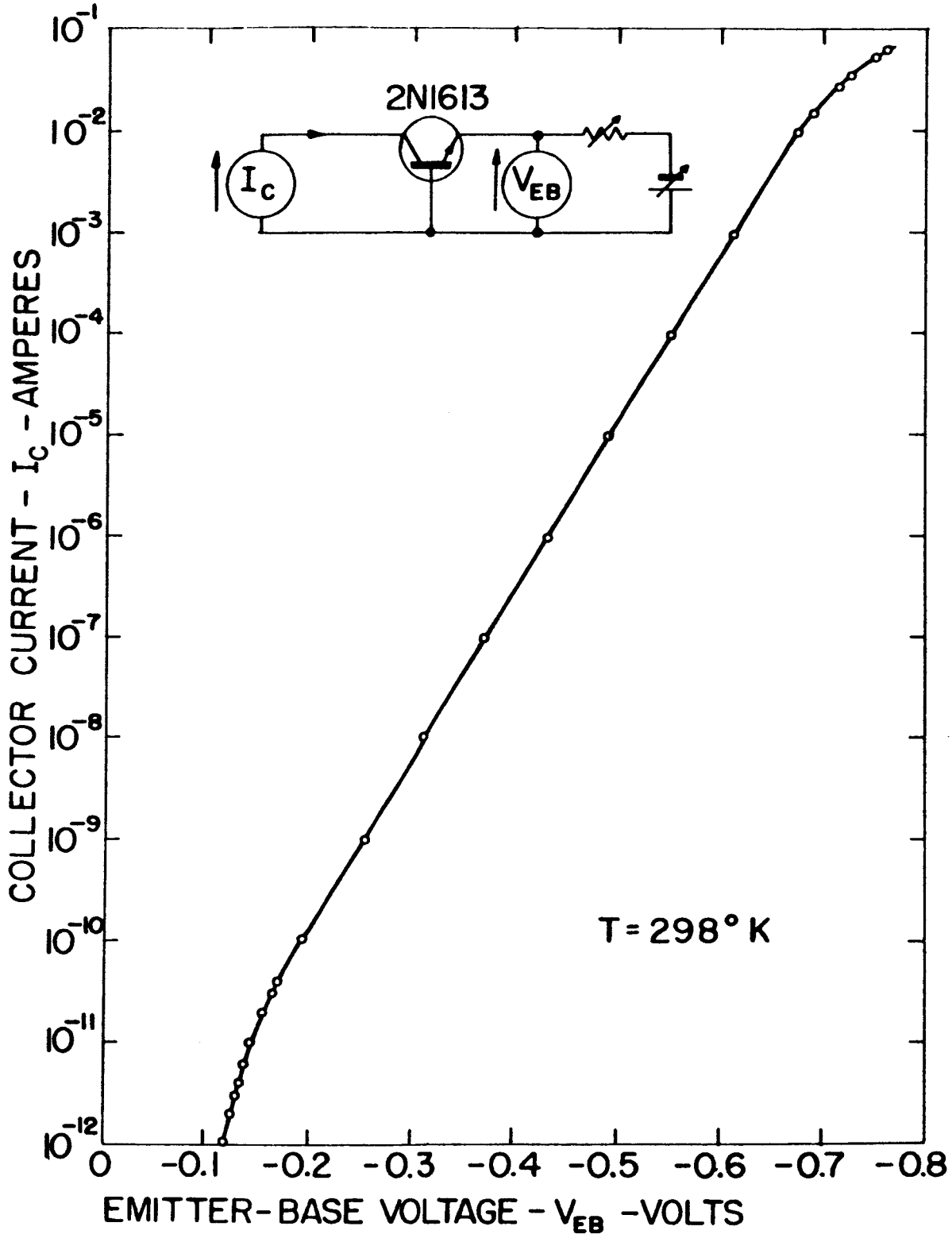

FIG. 14 - LOG I_c - V_{EB} CHARACTERISTIC FOR A 2N1613

range than the 2 or 3 decades needed here (fig. 14). A switch-selected decoder provides a pulse which is coincident with the selected line, and is picked to be above the meniscus of the cell: this line is the reference, or zero-absorption, line. The logged video during the reference line is switched (Q_1 is open, Q_2 and Q_3 are closed) to the capacitor across the positive input of a differential operational amplifier. During the rest of the frame time Q_1 is closed, and Q_2 and Q_3 are open, so that the logged video from the signal (non-reference) lines is applied to the negative input of the differential amplifier, which is scaled to have the same magnitude of gain from each input. Thus, the output is proportional to the difference between the log of the reference and log of the signal; this difference is equal to the log of the ratio of signal to reference, which is proportional to the optical density of the sample.

5.3 Uses of Television Equipment

The television equipment has been used as a scanning densitometer, with film input, in two modes: delay-line memory and holding-capacitor memory. The holding-capacitor technique clearly presents fewer problems and greater flexibility.

For use in the Model E, the standard u.v. source (a H85A3 lamp) is being replaced with a PEK Labs 109 super-high-pressure short-arc mercury lamp, which provides several times as much output in the 254 millimicrons band, and has a much smaller source area - and hence sharper images.

Storage properties of a vidicon have been looked into briefly, using the frame-blanking mode. The need for a monitor capable of operating at the camer's non-standard rates and rasters became apparent, and a standard oscilloscope has been modified for this purpose. Some of the refinements mentioned above will also help in making the storage measurements. Measurements will be made on several visible, ultraviolet, and slow-scan vidicons.

5.4 Microbe Identification

Ultraviolet absorption microphotographs of microbes and controls (dirt) have been taken and sent to Philco's Artificial Intelligence Department at Blue Bell, Pennsylvania where they will be processed on the IMITAC equipment. This equipment amounts to a fascimile transmitter and receiver, with an analog-to-digital converter at the transmitter, a digital-to-analog converter at the receiver, and one of their 2000 computers in between. This set-up permits many image-processing experiments to be readily performed. Two variables are being investigated in this experiment: number of lines of scan, and number of bits of gray-scale coding. We furnished photographic copy at various magnifications in order to vary the number of lines across a microbe, and they are varying the number of bits of gray scale (from silhouette to 6 bits). The resultant photographs will be examined to give an experimental determination of the information content necessary for microbe identification by skilled observers.

6. Computer Technology

We have become increasingly interested in exploiting the possibilities of computer technology as applied to the problems of exobiology. Our interest is several-fold. Firstly, to utilize computers as an aid in the engineering development of prototypes of new instruments by programming a general-purpose computer to set up the control and signal-processing systems, instead of investing in new construction for each new experimental situation. Secondly, to take advantage of the computer as a part of the ultimate instrument itself to add a new dimension to the utility of present techniques in, for example, various fields of spectroscopic analysis. Thirdly, we are interested in studying the fundamental questions of artificial intelligence, particularly those that relate to exobiology missions. What is the proper distribution of artificial and human intelligence which exploits the best features of both in experimental systems whose parts are separated by distances, as in the case of Mars, of about 40×10^6 miles?

Steps have been taken to implement these interests. We have made very fruitful contacts with the Stanford Computation Center. In particular we have completed arrangement to share access to a PDP-1 computer. Professor J. McCarthy of the Stanford faculty in computer science, who is responsible for this activity, is also interested in questions of artificial intelligence. We have applied for and been selected to receive one of the computers in the LINC Evaluation program. Our thinking on this subject is presented by a report entitled "An Instrumentation Crisis in Biology" prepared for other purposes and included as appendix C.

7. Personnel and Organization

The present organization and staff of the Instrumentation Research Laboratory is indicated below.

Principal Investigator	Professor J. Lederberg
Program Director	Dr. E. Levinthal
Biochemistry Group	Dr. Elie Shneour (Oct. 1962) Dr. John Westley (Sept. 1963) Jerry Lundstrom
Electrical Engineering Group	Harrison Horn Lee Hundley Nicholas Weizades (Nov. 1962)
Physics/Mathematics Group	Dr. M. Mandel (April 1963)

Three additional supporting personnel full-time.

During the past year the following papers have been presented by members of the laboratory.

E.C. Levinthal	-	"Detection of Extraterrestrial Life" Professional and Technical Group of Instrumentation and Measurements of the I.E.E.E. April, 1963
E. Shneour	-	"Instrumented Life Detection Devices" Jet Propulsion Laboratory Symposium on Current Research in Exobiology. Feb. 1963.
L. Hundley	-	"A Life Detection System for Mars Probes" "Instrument Society of America. March 1963.
J.F. Gibbons & H.S. Horn	-	"A Circuit with Logarithmic Transfer Response over 9 Decades" Solid-State Circuits Conference. Feb. 1963.

The following curricula vitae are for Drs. Shneour, Westley, Mandel and Mr. Veizades.

Elie A. Shneour

Education:

Ph.D.	California at Los Angeles	1958	Physiological Chemistry
M.A.	California at Berkeley	1955	Biochemistry
B.A.	Columbia	1947	Organic Chemistry, Physics

Training in electronics, theoretical and applied for research instrumentation.

Honors:

John Bard Scholar (1946) for excellence in chemistry and mathematics.
William Lockwood Prize (1947) for intellectual leadership on the campus.

Experience:

1960 to date: Research Associate in Chemistry, Lawrence Radiation Laboratory University of California at Berkeley. Advanced Fellow of the American Heart Association. Independent research on carotenoid pigments in photosynthesis. Involves use of chromatography, electrophoresis, radioisotopes, stable nuclides, mass and nuclear activation analyses, and classical techniques in physical, chemical and biological investigations.

1959-1960: Research Associate in Bacteriology, University of California at Berkeley. Fellow of the American Heart Association. Independent research on carotenoid metabolism in photosynthetic bacteria. Involved use of microbiological and biochemical techniques, including enzymology and mutant technology.

1958-1959: Research Fellow in Biochemistry, New York University Medical Center. Fellow of the American Heart Association. Supervised research on the metabolism of amino acids in bacteria and its relationship to bacterial viruses. Involved use of microbiological and biochemical techniques, including enzymology and mutant technology of bacteria and bacterial viruses.

1953-1958: Teaching and Research Fellow in Biochemistry (Berkeley 1953-1955) and Physiological Chemistry (Los Angeles 1955-1958). Fellow of the National Cancer Institute, National Institutes of Health. Included the initiation and presentation of a course entitled "Electronics for Biological Research" (Los Angeles, 1957)

1947-1953: Temporary appointment as Teaching Fellow in Chemistry and Physics, Bard College, New York (1947). Chemist, then Assistant Director of R. and D., J.A.E. Color Works, New York (1948-9). Senior Research Technician, Dept. of Agriculture, University of California at Berkeley (1950-3).

Important Publications:

"The Biosynthesis of Lycopene in Tomato Homogenates"
Elie Shneour & Irving Zabin
Journal of Biological Chemistry 234, 770 (1959)

"The Preparation and Characterization of Di-O-benzylidene-(-)-inositol"
Elie A. Shneour & Clinton C. Ballou
J. of the Amer. Chem. Soc. 80, 3960 (1958)

"On the Biosynthetic Relationship between Lycopene & Colorless Polyenes in Tomatoes"
Elie A. Shneour & Irving Zabin
Journal of Biological Chemistry 226, 861 (1957)

"Effect of Dietary Sterols and Sterol Esters on Plasma and Liver Cholesterol" (...)
D.W. Peterson, Elie A. Shneour & N.F. Peek
Journal of Nutrition 53, 451 (1954)
"
"Dietary Constituents affecting Plasma and Liver Cholesterol in Cholesterol-fed chicks"
D.W. Peterson, Elie A. Shneour, N.F. Peek & H.W. Gaffey
Journal of Nutrition 50, 191 (1953)

"Some Relationships among Dietary Sterols, Plasma and Liver Cholesterol Levels" (...)
D.W. Peterson, C.W. Nichols Jr. and Elie A. Shneour
Journal of Nutrition 47, 57 (1952)

"Carotenoid Pigment Conversion in Rhodopseudomonas spheroides"
Elie A. Shneour
Biochimica et Biophysica Acta 62, 534 (1962)

"Scintillation Counting of Solutions containing Carotenoids and Chlorophylls"
Elie A. Shneour, S. Aronoff & M.R. Kirk
Int. J. Appl. Rad. and Isotopes 13, 623 (1962)

"The Source of Oxygen in Rhodopseudomonas spheroides pigment conversion"
Elie A. Shneour
Biochimica et Biophysica Acta 65, 510 (1962)

"Isotopic Oxygen Incorporation in Xanthophylls of Spinacea oleracea quantasomes"
Elie Shneour & Melvin Calvin
Nature 196, 439 (1962)

"On the Use of the Nuclear Activation Reaction O^{18} (α,n)Ne^{21} for O^{18} Analysis"
E.A. Shneour
U.C.R.L. Reports (Atomic Energy Commission) 1034, 49 (1962)

"A Study of light-catalyzed Hydrogen Transport under Photosynthetic Conditions"
E.A. Shneour
Part I U.C.R.L. (AEC) 9652, 99 (1961)
Part II U.C.R.L. (AEC) 9900, 26 (1961)

John Westley

Education:

B.Sc.	University of Nottingham	1958	First Class Honors Degree in Organic Chemistry
Ph.D.	University of Nottingham	1961	Organic Chemistry

Honors:

Boots Research Fellowship (to carry out work on Ph.D. for 3 years)

Experience:

1962 up to date: Research Associate with Dr. J.S. Craig, San Francisco Medical Center, School of Pharmacy.

1961-1962: Research Associate with Dr. C. Djerassi, Chemistry Department, Stanford University. Work on Nystatin.

Sept. 1962: Presented a paper at the A.C.S. Meetings in Atlantic City entitled: A. Biosynthetic Approach to Structure Determination--Nystatin.

Publications:

Chemistry of Streptothricin and Related Antibiotics. Published May, 1962, Journal of the Chemical Society, 1642-1655

A joint paper with Dr. Rickards of Manchester University, England, to be published in the Journal of the Chemical Society - preliminary note in Tetrahedron Letters.

Paper in preparation with Dr. J.S. Craig for the Journal of the American Chemical Society on preparing Acetylenic Analogs of Stilboestrol.

July, 1963: Presenting a paper to the American Pharmacognosy Society in North Carolina on Biosynthesis-Nystatin.

Morton Mandel

Education:

B.C.E.	City College of New York	1944	
M.S.	Columbia University	1949	Engineering
Ph.D.	Columbia University	1957	Physics

Honors:

Sigma Xi, Tau Beta Pi, New York State Regents Scholarship
New York State War Veterans Scholarship

Experience:

Masters thesis on the use of finite difference methods in solving structural problems. Ph.D. thesis in microwave spectroscopy of gases. Instructor in the Civil Engineering Department, Stevens Institute of Technology.

March 1963 to date: Research Physicist. Genetics Department, Stanford University.

1961-1962: Engaged in magnetic resonance studies of solids at the General Telephone and Electronics Laboratories.

1957-1961: Research Associate and Assistant Professor, Physics Department, Stanford University. Investigated magnetic phenomena such as susceptibilities, spin specific heat, exchange interactions and relaxation times in free radical systems. During these same years served as a consultant to the Fairchild Semiconductor Corporations, Rheem Semiconductor Corporation, and Hewlett Packard Company.

1956-1957: Member of the Technical Staff, Bell Telephone Laboratories, engaged in diffusion studies in silicon and semiconductor device work.

1952-1956: Research Assistant in Columbia University Radiation Laboratory testing and developing microwave components.

1949-1950 Engineer for Standard-Vacuum Oil Company.

Publications:

"Microwave Spectra in CsF, CsCl, and CsBr", Phys. Rev. 92, 901 (1953).

"Microwave Spectra of the Alkali Halides," Phys. Rev. 96, 629 (1954).

"Microwave Spectra of the Tl, In, and Ga Halides," Phys. Rev. 109, 1572 (1958).

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"Lattice Heating Effects in Spin-Lattice Relaxation Time Measurements," Bull. Amer. Phys. Soc., Series II, 6, 141 (1961).

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Invited Paper: American Chemical Society, "The Nature of the Chemical Bond in the Diatomic Gallium, Indium, and Thallium Halides, March 1958.

Nicholas Veizades

Education:

B.S.	University of California, Berkeley	1954-1958	Electrical Engineering
M.S.	Stanford University, Stanford	1959-1961	Engineering Science

Experience:

1962 up to date: Electrical Engineer in the Department of Genetics at Stanford University

1961-1962: Project Engineer at the Fairchild Instrument and Camera Co., Palo Alto.

1958-1961: Senior Engineer at the Link Division of General Precision Inc., Palo Alto.

Special Field: Electronic Circuit Design

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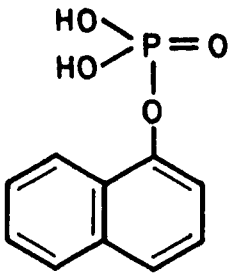
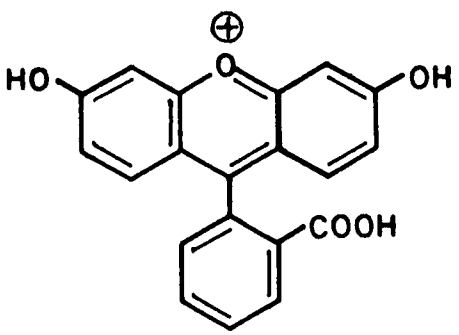
Glossary of Organic Compounds

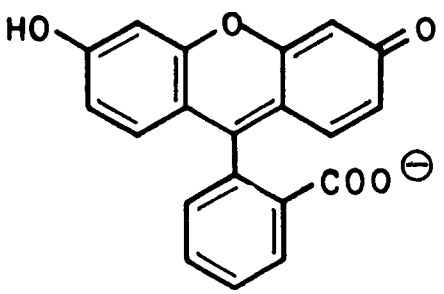


I α- naphthyl phosphate



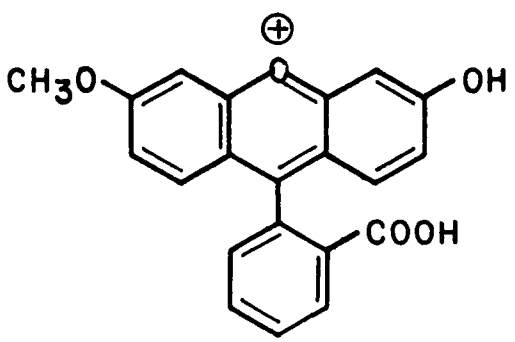
II 3,6-fluorandiol

(Fluorescein)



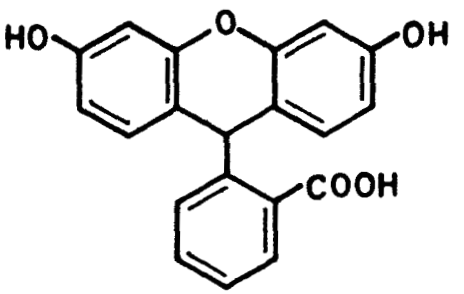
III 9-(o-carboxyphenyl)-6-hydroxy-3-isoxanthenone

(Uranine)



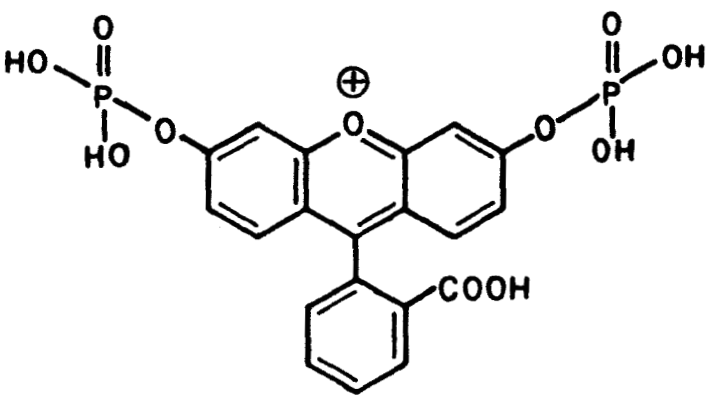
IV 3-methoxyfluoran-6-ol

(Fluorescein monomethyl ether)



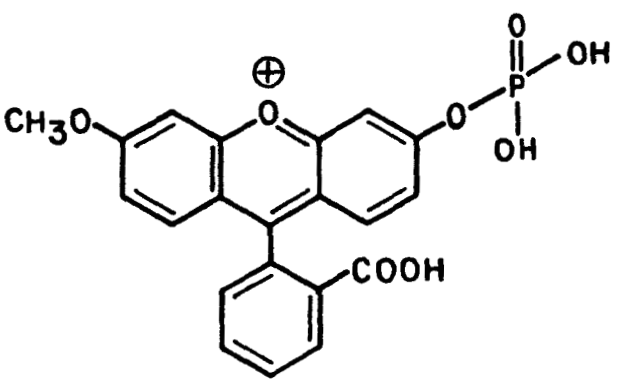
V dihydroxyfluoran-3,6-diol

(Fluorescin)



VI 3,6-fluorandiol-3,6-diphosphate

(Fluorescein diphosphate)



VII 3-methoxyfluoran-6-ol-phosphate

(Fluorescein monomethyl ether monophosphate)